



WHO International Standard
6th International Standard 2025 Thromboplastin, Human,
Recombinant, Plain
NIBSC code: 24/114
Instructions for use
(Version 2.0, Dated 11/02/2026)

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1. INTENDED USE

This standard is used for the ISI calibration of thromboplastin reagents [1]. The standard was established as the WHO 6th International Standard (IS) for Thromboplastin Human, Recombinant, Plain by the WHO Expert Committee on Biological Standardization (ECBS) in 2025 and consists of ampoules containing lyophilised recombinant human tissue factor reagent (coded 24/114). Details of the collaborative study can be found in document WHO/BS/2025.2491.

2. CAUTION

This preparation is not for administration to humans or animals in the human food chain.

This product contains recombinant human tissue factor produced in *E. coli* and synthetic phospholipids. It does not contain human or bovine-derived thromboplastin. However, it may contain bovine serum albumin as a stabilizer. As with all materials of biological origin, this preparation should be regarded as potentially hazardous to health. It should be used and discarded according to your own laboratory's safety procedures. Such safety procedures should include the wearing of protective gloves and avoiding the generation of aerosols. Care should be exercised in opening ampoules or vials, to avoid cuts.

3. UNITAGE

INTERNATIONAL SENSITIVITY INDEX (ISI) AND COLLABORATIVE STUDY

The International Standard has been assigned an ISI value of 1.08.

The ISI value was determined in a collaborative study against the 5th WHO International Standard for tissue factor from human origin (rTF/16). The study involved 4 calibration laboratories (total 8 operators). The candidate 6th International Standard (24/114) and the WHO 5th International Standard Thromboplastin Human, Recombinant plain (rTF/16) were tested in each laboratory by two expert operators using the harmonised Manual Tilt Tube technique [2,3]. Test plasmas were freshly prepared from healthy subjects and patients on long term vitamin K antagonist therapy. Participants selected patient plasmas with prothrombin times (PT) corresponding to an interval of International Normalized Ratios (INR) from 1.5 to 4.0 [4]. To account for the effect of inter-day variation, PT measurements were performed in each laboratory on ten different days (not necessarily consecutive). Participants included on each day plasmas from 2 healthy individuals and 6 anticoagulated patients, using plasmas of different healthy subjects and patients on each working day. To minimize the effect of possible plasma instability on the prothrombin times, the order was changed each day. All plasmas need to be tested with the first thromboplastin before proceeding to the second thromboplastin. Plasmas were tested on each day according to the following order:

- normal plasma 1, patient plasma 1 through 6 and normal plasma 2.

4. CONTENTS

Country of origin of biological material: Germany.

THROMBOPLASTIN REAGENT (lyophilised portion coded 24/114), the residue of a solution containing:
Tissue Factor (TF). A human recombinant membrane-spanning protein, expressed in *E. coli*, combined with synthetic phospholipids, calcium, buffers, and stabilizers

5. STORAGE

Unopened ampoules of lyophilised reagent (24/114) should be stored in the dark at -20 °C or below.

Please note: NIBSC may ship these materials with cooling packs to ensure stability in transit.

6. DIRECTIONS FOR OPENING

DIN ampoules have an 'easy-open' coloured stress point, where the narrow ampoule stem joins the wider ampoule body. Various types of ampoule breaker are available commercially. To open the ampoule, tap the ampoule gently to collect material at the bottom (labelled) end and follow manufactures instructions provided with the ampoule breaker.

7. USE OF MATERIAL

No attempt should be made to weigh out any portion of the freeze-dried material prior to reconstitution

Equilibrate ampoules at room temperature for at least 20 minutes before reconstitution.

Each ampoule of the lyophilised reagent (24/114) is to be reconstituted with exactly 2.0 ml of purified water.

Do not attempt to mix the contents by placing the thumb over the open end of the ampoule.

Leave the ampoule 24/114 undisturbed for 60 minutes at room temperature and swirl gently to dissolve the contents. Ensure that the entire lyophilised reagent is dissolved. Pool the contents of the ampoules if more than one is needed to complete one calibration session. Leave the reconstituted thromboplastin (24/114) at room temperature and use within 2 hours of reconstitution. This is in a time period of 60-180 minutes. Unused material should be discarded.

CALIBRATION PROCEDURE TO BE USED WITH INTERNATIONAL STANDARD 24/114

According to the WHO Guidelines [5] calibration of thromboplastins should be performed on plasmas from 20 healthy subjects and 60 patients on stabilized long term vitamin K antagonist therapy. The whole calibration procedure can be conveniently split into five or more working sessions, not necessarily consecutive.

Schedule of one-day calibration:

During the first 2 hours collect the blood, centrifuge and separate the platelet-poor plasma, and reconstitute thromboplastins according to the instructions. During the next 2 hours perform the actual testing of plasmas according to the design provided (see below).

Selection of healthy subjects and patients

Healthy subjects must be ambulant adults (females taking oral contraceptives can be included). On each working day use male and female subjects (if it is possible) and select different subjects each day. Patients must be different on each day and chosen among those who are in good health (outpatients) and have been stabilized for at least 6 weeks in the range of treatment between 1.5 and 4.0 INR, according to the routine reagent of the laboratory. Select patients covering the whole range of anticoagulation from 1.5 to 4.0. To avoid bias all results obtained with the chosen patients' plasmas must be recorded.



Blood collection and plasma preparation

At the beginning of each working day collect blood from healthy subjects and patients stabilized on long term vitamin K antagonist therapy. Blood will be collected by clean venipuncture in a plastic (or glass siliconized vacuum) tube containing 0.105 - 0.109 mol/L (3.1-3.2 (g/v)%) of the dihydrate form trisodium citrate (9 volumes of blood/1 volume of sodium citrate anticoagulant) [6, 7]. The tube must be inverted several times to ensure complete mixing of blood and anticoagulant. Citrated blood will be centrifuged as soon as possible after collection; at least 2500 g for 10 minutes at room temperature. Platelet-poor plasmas are transferred into plastic tubes (composed of polypropylene) and stored capped at room temperature, until tested.

Preparation of thromboplastins

On each working day:

Equilibrate a suitable number of ampoules of lyophilised reagent (24/114) at room temperature for at least 20 minutes before reconstitution.

Reconstitute ampoules of lyophilised reagent (24/114) following instructions (see sections 6 and 7 above).

Discard the remaining reconstituted thromboplastins at the end of each working day.

Testing procedure

If testing is performed in 10 working sessions, the following procedure is used in each session. Test the 8 plasma samples (2 normal and 6 patients stabilized on long term vitamin K antagonist therapy) with the two thromboplastins according to the design given below. Testing must be done as single determinations. Measurements using 24/114 must be performed exclusively by using the harmonized Manual (Tilt Tube) technique, whereas a coagulometer may be used for testing with the thromboplastin to be calibrated. To avoid prolonged removal of tubes from the water, the use of an illuminated water-bath is recommended. See below for the actual testing procedure of the harmonized Manual Tilt Tube technique. The order of testing normal and patient plasmas will be random and must reflect the order of blood collection if this is considered random. As an example collect first the normal 1 (which will be tested first) then the 6 patients on long term vitamin K antagonist therapy and finally the normal 2 (which will be tested last). In any case the order of testing should not be related to the prolongation of the clotting time of the patient plasma. The order of testing on each working day shall be as follows:

Normal 1 Patient 1 Patient 2 Patient 3 Patient 4 Patient 5
Patient 6 Normal 2

If testing is performed in 5 working sessions, the number of samples used in each session is 16 (4 normal and 12 patients). The order of testing on each working day shall be as follows:

Normal 1, Normal 2, Patient 1 - 12, Normal 3, Normal 4

All plasmas shall be tested with the first thromboplastin before proceeding to the second thromboplastin if both are used with the harmonized Manual Tilt Tube technique. The order of thromboplastins shall be alternated from day to day. The same expert operator shall be in charge to carry out the whole calibration.

Actual testing with 24/114

- Take care that the actual room temperature is 20-22 °C (RT).
- Fill the water level in the water bath, i.e. 1 cm underneath the edge of the bath (harmonization of distance of tube's horizontal position to water bath during tilting, i.e. 2 – 10 cm).

- Keep empty test tubes in a vertical position in a rack in the water bath at 37 °C (± 0.1 °C) for at least 4 minutes at a depth of 3.5 cm before reagent and plasma samples are transferred successively with micropipettes.
- Mark the pipette at the level corresponding to the upper edge of the test tube to determine the exact level of pipetting the plasma, i.e. 1 cm above the thromboplastin reagent in the tube.
- Harmonize the speed of pipetting the reagent and plasma into the glass tube (avoid splashing by letting the tip of pipette rest against the wall of the tube); the stop watch must be started when the operator begins to push the button of the pipette; the time of the complete push movement being 0.5 s.
- Pipette 200 μ L of thromboplastin into glass test tube at 37°C and incubate for 2 minutes.
- Pipette 100 μ L of not prewarmed citrate plasma 1 cm above the level of thromboplastin with the tip resting against the wall of the tube and start the stopwatch with the other hand immediately.
- After pipetting the plasma into the glass tube shake gently with the tube immersed in the water to mix the contents. Put the tube in the rack in the water bath and lay down the pipette.
- Take the tube out of the rack, the test tube shall be kept manually in the water covering 5 cm of the tube before starting the tilting (Figure 1A [2]).
- Let your hand rest on the edge of the water bath (harmonization of distance of tube's horizontal position to water bath during tilting, i.e. 2 – 10 cm).
- Start manual tilting of the tube 7 seconds after adding the plasma to the thromboplastin and starting the stopwatch.
- Tilt the tube through an angle of nearly 90° by taking the tube out of the water for 2 seconds and putting it back in the water for 1 second (Figure 1B [2]). The tube should not be stationary during this cycle but continuously tilted with the operator's hand resting on the edge of the water bath.
- Then the cycle shall be repeated until the clot is formed. In the horizontal position, the tube is kept not more than 10 cm and not <2 cm above the water level (Figure 1B [2]).
- Before the mixture clots, the operator observes the mixture flowing from the bottom to three-quarters of the length of the tube in the nearly horizontal position and back to the bottom.
- When clotting commences, the speed of flowing is reduced. At this point, the operator is keen to observe the final stopping of the flow.
- When flow is stopped, the operator stops the timer and records the clotting time in seconds in two decimal places.

Equipment

- Water bath at a constant temperature of 37 °C. Dimensions water bath: 40x30x20 cm. Water in the bath is continuously circulated by a pump.
 - An electric thermostat with a power of 1600 to 2000 W is used to keep the temperature at 37 °C (± 0.1 °C)
 - Light source (minimum 40 W) mounted 20 cm above the water level to illuminate the test tube
 - Test tube rack (custom made)
 - Nonsiliconized glass tubes (dimensions: 75x12 mm ad wall thickness 0.8 mm). Tubes are made of borosilicate glass
 - Air displacement micropipettes for reagent and plasma samples, yearly calibrated according to guideline. Using interchangeable plastic tips.
 - Digital electronic 60 Memory Stopwatch, traceable to the National Institute for Standards and Technology, in compliance with ISO 9001 ISO/IEC 17025 AND ANSI/NCSLZ540-1
- Statistical analysis and ISI determination
For statistical analysis and ISI determination refer to the WHO Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists [5]. These requirements are available



on request from the Biologicals Unit, WHO, CH-1211 Geneva 27, Switzerland.

8. STABILITY (Add or amend as necessary)

Reference materials are held at NIBSC within assured temperature-controlled storage facilities and they should be stored on receipt as indicated on the label. It is the policy of WHO not to assign an expiry date to their international reference materials. Stability studies have indicated that this material is suitably stable, when stored at -20 °C or below, for the assigned values to remain valid until the material is withdrawn or replaced [8]. These studies have also shown that the material is suitably stable for shipment with cooling packs without any effect on the assigned values. Users who have data supporting any deterioration in the characteristics of any reference preparation are encouraged to contact NIBSC.

9. REFERENCES

- van den Besselaar, A.M.H.P., et al., Defining a metrologically traceable and sustainable calibration hierarchy of international normalized ratio for monitoring of vitamin K antagonist treatment in accordance with International Organization for Standardization (ISO) 17511:2020 standard: communication from the International Federation of Clinical Chemistry and Laboratory Medicine–SSC/ISTH working group on prothrombin time/international normalized ratio standardization. *Journal of Thrombosis and Haemostasis*, 2024. 22(4): p. 1236-1248.
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- WHO Expert Committee on Biological Standardization. Guidelines for thromboplastins and plasma used to control oral anticoagulant therapy with vitamin K antagonists. WHO Technical Report Series 2013; No. 979: 271-316.
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- Kitchen, S, et al., International Council for Standardisation in Haematology (ICSH) recommendations for collection of blood samples for coagulation testing. *Int J Lab Hematol*. 2021; 43: 571–580.
- Van den Besselaar AMHP, Abdoel CF, Hubbard AR. Long-term stability of international standards for thromboplastin stored at -20 °C, -70 °C, and -150 °C. *Thromb Res* 2015;164-167.

10. ACKNOWLEDGEMENTS

Grateful acknowledgements are due to the participants in the collaborative study. This study was organized and carried out under the auspices of the Scientific and Standardization Committee (SSC) (Subcommittee on Control of Anticoagulation), of the International Society on Thrombosis and Haemostasis (ISTH). Grateful acknowledgements are also due to Siemens (Marburg, Germany) and Technoclone GmbH (Vienna, Austria), who donated the candidate materials and control samples for the collaborative study.

11. FURTHER INFORMATION

Further information can be obtained as follows;
This material: enquiries@nibsc.org
WHO Biological Standards:
<http://www.who.int/biologicals/en/>
JCTLM Higher order reference materials:
<http://www.bipm.org/en/committees/jc/jctlm/>
Derivation of International Units:
http://www.nibsc.org/standardisation/international_standards.aspx
Ordering standards from NIBSC:
<http://www.nibsc.org/products/ordering.aspx>
NIBSC Terms & Conditions:
http://www.nibsc.org/terms_and_conditions.aspx

12. CUSTOMER FEEDBACK

Customers are encouraged to provide feedback on the suitability or use of the material provided or other aspects of our service. Please send any comments to enquiries@nibsc.org

13. CITATION

In all publications, including data sheets, in which this material is referenced, it is important that the preparation's title, its status, the NIBSC code number, and the name and address of NIBSC are cited and cited correctly.

14. MATERIAL SAFETY SHEET (Add or amend as necessary)

Classification in accordance with Directive 2000/54/EC, Regulation (EC) No 1272/2008: Not applicable or not classified

Physical and Chemical properties	
Physical appearance: Freeze-dried powder	Corrosive: No
Stable: Yes	Oxidising: No
Hygroscopic: Yes	Irritant: No
Flammable: No	Handling: See caution, Section 2
Other (specify): Contains recombinant protein, stabilizers and preservative (azide)	
Toxicological properties	
Effects of inhalation:	Not established, avoid inhalation
Effects of ingestion:	Not established, avoid ingestion
Effects of skin absorption:	Not established, avoid contact with skin
Suggested First Aid	
Inhalation:	Seek medical advice
Ingestion:	Seek medical advice
Contact with eyes:	Wash with copious amounts of water. Seek medical advice
Contact with skin:	Wash thoroughly with water.



Action on Spillage and Method of Disposal

Spillage of ampoule contents should be taken up with absorbent material wetted with an appropriate disinfectant. Rinse area with an appropriate disinfectant followed by water. Absorbent materials used to treat spillage should be treated as biological waste.

15. LIABILITY AND LOSS

In the event that this document is translated into another language, the English language version shall prevail in the event of any inconsistencies between the documents.

Unless expressly stated otherwise by NIBSC, NIBSC's Standard Terms and Conditions for the Supply of Materials (available at http://www.nibsc.org/About_Us/Terms_and_Conditions.aspx or upon request by the Recipient) ("Conditions") apply to the exclusion of all other terms and are hereby incorporated into this document by reference. The Recipient's attention is drawn in particular to the provisions of clause 11 of the Conditions.

16. INFORMATION FOR CUSTOMS USE ONLY

Country of origin for customs purposes*: UK * Defined as the country where the goods have been produced and/or sufficiently processed to be classed as originating from the country of supply, for example a change of state such as freeze-drying.
Net weight: 0.148 g
Toxicity Statement: Non-toxic
Veterinary certificate or other statement if applicable.
Attached: No

17. CERTIFICATE OF ANALYSIS

NIBSC does not provide a Certificate of Analysis for WHO Biological Reference Materials because they are internationally recognised primary reference materials fully described in the instructions for use. The reference materials are established according to the WHO Recommendations for the preparation, characterization and establishment of international and other biological reference standards [https://www.who.int/publications/m/item/annex2-trs932\(revised 2004\)](https://www.who.int/publications/m/item/annex2-trs932(revised%202004)). They are officially endorsed by the WHO Expert Committee on Biological Standardization (ECBS) based on the report of the international collaborative study which established their suitability for the intended use.